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Utilization of seafood processing wastes for cultivation of the edible mushroom *Pleurotus flabellatus*

Subbu lakshmi, S.[1*] and Sornaraj, R.[2]

[1]Department of Microbiology, Kamaraj college, Tuticorin-628003, Tamil Nadu, India.
[2]PG & Research Department of Zoology, Kamaraj College, Tuticorin-628003, Tamil Nadu India.

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A study was conducted to examine the utilization of seafood processing wastes for artificial cultivation of edible mushroom *Pleurotus flabellatus* in laboratory condition. Utilizing bioconversion technology such as the slow release of nutrients for agricultural based activities like producing mushroom will profitably reduce seafood waste and also enhancing environmental quality. The selected agro-industrial wastes such as coir pith, woodchips and sugarcane bagasse were mixed with cooked fish waste (CFW) in the ratio of 1:1 (500 g : 500 g). The substrates which were not mixed with CFW were treated as control. All the above materials were allowed to decompose partially for about 15 days. The partially composted materials were placed in heat resistant transparent sterilized polyethylene bags. Each sterile bag was then aseptically inoculated with *P. flabellatus*. The bags were then incubated under ambient temperature and controlled humidity. The maximum biological yield per bed was obtained with sugarcane bagasse control bed 58.05±0.88 g/bed. The lowest yield was observed in the substrate woodchips: CFW (1:1) 24.43±0.30 g / bed. Based on the mass obtained for *P. flabellatus*, the best substrates were in the ordered of woodchips>coir pith>sugarcane. This could be used to cultivate an edible mushroom while at the same time promoting environmental sustainability and increase soil fertility.

Key words: Mushroom, cooked fishery waste, solid substrates, biological yield.

INTRODUCTION

Seafood processing activities have raised serious waste production and disposal concerns all over the globe. Commercially, fishing and aquaculture usually generate large amounts of waste that must be disposed (Burrows et al., 2007). In Korea, many restaurants specialize in sliced raw fish, and large amounts (approximately 2,100 t/day) of fish waste are generated every day (Kim et al., 2010). Fish consumption continues to increase steadily worldwide and seafood is gaining in popularity because of its health benefits. At the same time, large amounts of fish waste are being generated, mostly from the industrial processing of fish. These large quantities of fish waste

*Corresponding author. E-mail: daisyrani11@yahoo.com.

have not been utilized efficiently, and the disposal of fish waste can have large negative impacts on local environments. Unutilized fish waste is often disposed of by landfill or incineration, or by dumping into the sea. Therefore, there is an urgent need to find ecologically acceptable means for reutilizing fish waste (Joong, 2011).

The fermented broth of fisheries waste could be a valuable resource for agriculture. Recently, some studies have examined the reutilization of biodegraded fisheries-waste products as liquid fertilizer (Kim and Lee, 2009; Kim et al., 2010; Dao and Kim, 2011). Fish sludge contains macro and micro nutrients, especially high levels of nitrogen and phosphorus. Sewage sludge mixed with different organic waste materials is now usual in composting experiments (Roca-Perez et al., 2009).

The "Mushroom" word is used in all part of world to describe the fruiting bodies of saprophytic, mycorrhizal and parasites fungi, belonging to the order of Basidiomycetes or Ascomycetes. They can be found in soils rich in organic matter and humus, moist wood, animal waste, etc. The cultivation of edible mushrooms is actually an alternative biotech which is fast, environmentally friendly and feasible to recycle organic byproducts from agribusiness into high nutritional and medicinal quality food both with respect to the amount of protein or minerals and selected substances with medicinal and pharmacological properties, for example the presence of β-glucans like lentinan, and thus it can contribute significantly in feeding human. (Diego Cunha Zied et al*.,* 2011).

Cultivation of edible mushroom using various wastes is an alternative for solid waste management. Mushrooms have very high nutritional value. It is rich in protein, vitamins and minerals. More than 2000 species of fungi are reported edible throughout the world and 200 belonging to 70 genera are reported from India. Of these, about 80 distinct varieties which are edible are sold in various countries. Presently, only three mushrooms namely *Agaricus bisporus* (white button mushroom), *Pleurotus* spp. (oyster mushroom) and *Volvariella volvacea* (Paddy straw mushroom) are under commercial cultivation in India. *A. bisporus* with its temperature preference could not be cultivated in tropical and sub tropical situations leaving the choice to only *Pleurotus* spp. (Renganatha et al*.,* 2008).

Pleurotus species are rich source of proteins and an abundance of essential amino acids, minerals (Ca, P, K, Fe, Na) and also contain vitamins C, B-complex – thiamine, riboflavin, niacin and folic acid (ÇaglarIrmak, 2007; Regula and Siwulski, 2007). Oyster mushroom *Pleurotus ostreatus*, due to its documented probiotic properties and relatively high nutritive value, are recommended in numerous countries as an addition to the daily diet (Bernas et al., 2006).

Successful utilization of agro-wastes for both mycelial and sporophore formation of macrofungi, supplies the nutrients needed by these fungi to convert them to protein-rich palatable food. It also helps in reducing the environmental and health hazards posed by indiscriminate dumping of the wastes (Pandey, 2006). Mushroom hyphae secrete large amounts of extracellular enzymes which bring about the degradation of macromolecules such as cellulose, hemicellulose, lignin and protein in the substrates (Narsi et al., 2006; Kuforiji and Fasidi, 2008).

Oyster mushroom can be grown on various substrates (Hassan et al., 2011). Cultivation of edible mushrooms is a biotechnological process for lignocellulosic organic waste recycling. Roughly 300 mushrooms species are edible, but only 30 have been domesticated and ten grown commercially (Barny, 2009). *Pleurotus* sp. is the second most cultivated edible mushroom worldwide after *Agaricus bisporus.* To date approximately 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently (Ruhl et al., 2008). These mushrooms have economical and ecological values and medicinal properties. They are able to colonize and degrade a large variety of lignocellulosic substrates and other wastes which are produced in agricultural, forest and food processing industries (Sanchez, 2010).

Pleurotus pulmonarius and *Pleurotus ostreatus*, exhibited strong anti-inflammatory and immunemodulatory properties due to their chemical composition (Lavi et al., 2010). In the present study using *Pleurotus flabellatus* an attempt has made to convert fishery waste in to the valuable organic manures.

MATERIALS AND METHODS

Tissue culture technique

A large healthy and fresh mushroom *P. flabellatus* were collected from the Agricultural College at Killikulam, Tirunelveli District, Tamil Nadu and India. It was cleaned with 75% alcohol. The mushroom was splitted in half by hand longitudinally and some inside tissue taken from the upper part of the stripe. It was placed centrally on the surface of the sterilized potato dextrose agar with a sterilized needle and kept at room temperatures (28 ± 2°C) for eight days. Within two or three days some white, delicate mycelia was produced from the small piece of the tissue. About eight days later, the mycelium had grown rapidly and covered the surface of the agar medium. The growth of mycelium around the tissue inserted without contamination was considered as positive growth. The pure culture were collected and stored in the slants at 4°C for a period of a month. Then, it was ready to transfer to spawn substrate to make spawn. This spawn was used as inoculums for cultivation of mushroom (Stamets, 2000; Dhouib et al., 2005).

Spawn production

Spawn preparation was done using the *Shorgum vulgare* grains. The *S. vulgare* grains (1 kg) were placed in a trough of water to remove the chaff grains. Then, it was half cooked (~ 30 min). The excess water were drained and spread over a clean Hessian cloth. For every kilogram of grain, 20 g calcium carbonate was mixed so as to maintain the pH for the growth of the fungus. Moreover, the

calcium carbonate coating prevented the grains from sticking. These grains were filled in clean polythene bags (300 g/bag). Then, the bags were tightly plugged with nonabsorbent cotton and wrapped with paper, tied with a thread and placed in an autoclave for sterilization (20 lbs pressure for 2 h). After cooling, the bags were ready for inoculation. With the help of the gel puncher, a 10 mm diameter disc was made on the Petri plates having fully grown pure mycelium and transferred to the spawn bags. The bags were incubated at the room temperature. In about 15 days, the white colored mycelial growth was spread over the bag (Mane et al., 2007; Vetayasuporn et al., 2007; Royse et al., 2004; Shah et al., 2004; Baysal *et* al., 2003; Obodai et al., 2003a; Stamets, 2000). From this mother spawn, 30 first generation spawn were prepared. Each first generation bag having 10 g of mother spawn and from this second generation spawns were prepared.

Compost of fish waste

Fishery wastes were collected from the food processing unit at Thoothukudi District, which contains head, tail, shells, intestine, fins, dead fishes and so on. The wastes obtained were brought to the laboratory and the uncompostable materials such as shells and large bones were removed. The remaining wastes were cut into small uniform size pieces and allowed to cook for 15 min. Disease-free agro-industrial wastes (sugarcane baggase, coir pith and woodchips) were collected from Thoothukudi district which were cut in to small pieces (2 to 3 cm) and sun-dried in order to achieve proper drying. The selected agro-industrial wastes were mixed with cooked fish waste (CFW) in specific ratio 1:1 (500:500 g); while those which were not mixed with CFW are regarded as control. All the above materials (1:1 and control) were heaped in a separate plastic container and allowed to decompose for about 15 days. During the decomposition, water was sprayed over the materials with turning and restacking for every day to produce homogenize composed. Finally, the partially fish compost (15^{th} day compost) materials were used for mushroom cultivation.

Preparation of *P. flabellatus* bed

The partially fish compost (15^{th} day compost) materials were filled in polythene bags and sterilized at 121°C for 2 h. After sterilization, the bags were cooled to room temperature. Then polythene bag of 30×15 cm size was taken and the bottom of the bag was tied with a thread to provide a flat circular bottom to the mushroom beds. The partially fish compost (15^{th} day compost) materials were transferred to these polythene bags carefully along with previously prepared experimental spawns. The spawning was done in four to five layers. One bottle of spawn was used for two experimental bags. Then, the mouth of the bag was tied with the help of a twine and air holes were made on the sides of the bags for the free flow of air in to the mushroom bed. These polythene bags were kept in the dark room at 25 to 30°C. Relative humidity in the room was maintained as 86±4% and temperature as 26.5±0.05°C, respectively by pouring 25 L of water per day on the floor and on the walls. Whenever is necessary, the moisture content of the bags were maintained by the mist sprayers. The matured *Pleurotus* species fruiting bodies were identified by the formation of curl margin of the cap. It was then harvested from the root from the base by using a sharp sterilized knife. Mushroom matured generally 48 h after the appearance of primordia. Data on period of after completion of mycelium running, days of first harvest, number of fruiting bodies, length, diameter, biological yield, biological efficiency, moisture content of mushroom and dry yield were recorded. The biological efficiency (BE) percentage [fresh weight of harvested mushrooms/dry matter content of the substrate] x 100 (Royse et al., 2004; Stamets, 2000).

Data collection

Data on the following parameters were collected following the standard procedures (Ashrafuzzaman et al., 2009).

Time required for completion of mycelium running

Day required from opening to primordial initiation and days required from opening to harvesting on different substrates were recorded.

Number of fruiting body and dimension of pileus

Number of well-developed fruiting body was recorded. Dry and pinheaded fruiting body was discarded but twisted fruiting body was included during counting. Thickness of the pileus of four randomly selected fruiting bodies and diameter was recorded.

Biological yield (BY) and dry yield

Biological yield in g/500 g packet was recorded by weighing the whole cluster of fruiting body without removing the lower hard and dirty portion. Dry yield was recorded by weighing the fruiting bodies after drying.

Biological efficiency

Biological efficiency was calculated using the formula: [fresh weight of harvested mushrooms/dry matter content of the substrate] x 100.

Determination of moisture content

The moisture content of the compost was estimated by drying 2 g of fresh compost in an oven at 80°C for three consecutive days. It was cooled in a desiccator and weighed. The moisture content was calculated by the following formula.

Moisture content (%) = [(Fresh weight - dry weight) / fresh weight] x 100.

Statistical analysis

The experiment was done completely randomized design with three replications (n = 3). Data was analyzed and graph was constructed by Microsoft Excel.

RESULTS

Days to complete mycelium running

Days to complete mycelium running in mushroom bed ranged from 16 to 39 days on different agro-industrial waste (Figure 1). The lowest days to complete mycelium running was recorded on Wood chips control bed (16±1 days). The highest number of days required to complete

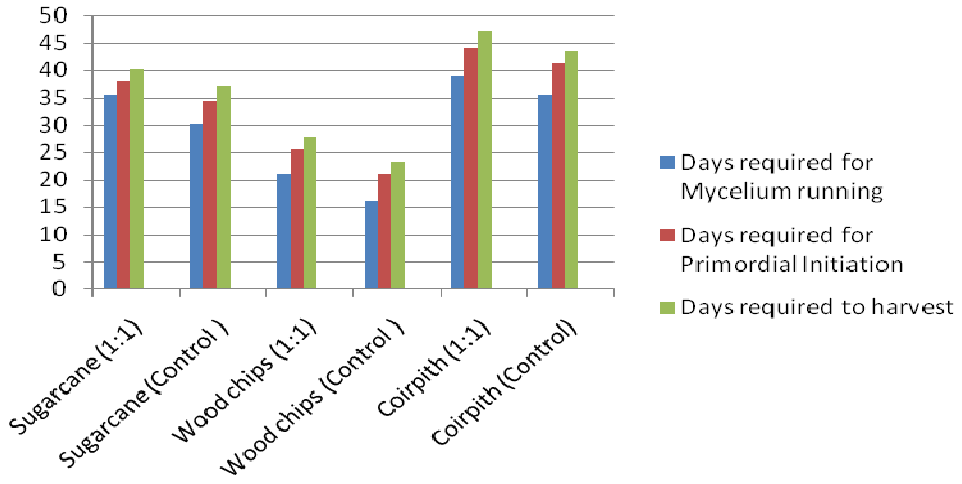

Figure 1. Time required for growth performance of *Pleurotus flabellatus*.

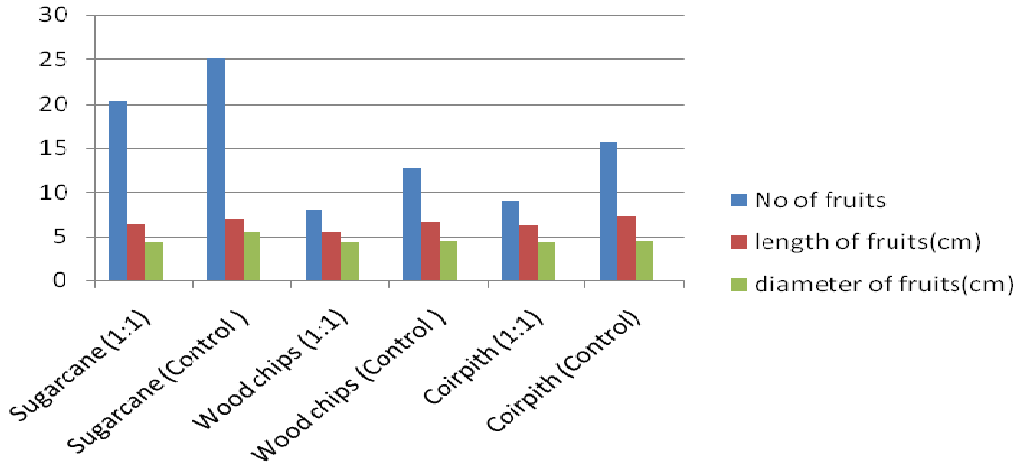

Figure 2. Effect of agro-industrial wastes on the development and size of fruiting bodies of *Pleurotus flabellatus.*

mycelium running was recorded with Coir pith: Cooked Fish Waste (1:1) bed (39±1 days).

Days required for primordial initiation and first harvest

Primordial initiation was minimum on wood chips control bed (21±1 days). The highest number of days required from opening to primordial initiation was on the coir pith : cooked fish waste (1:1) bed (44±1) followed by coir pith control bed (41.33±1.52). Days to first harvest ranged from 23.33±1.52 to 47.33±0.57 on different substrate. The maximum number of days required from opening to first harvest was recorded with coir pith: cooked fish waste (1:1) bed (47.33±0.57) (Figure 1).

Effective fruiting body per packet

Number of well-developed fruiting body was recorded and presented in Figure 2. Dry and pin headed fruiting body was discarded but twisted and tiny fruiting body was included during counting. The highest number of effective fruiting body was obtained from sugarcane control bed (25±2), while the lowest was obtained from control wood chips : cooked fish waste (1:1) bed (8±0.26).

Length of stalk and diameter of pileus

The highest length of stalk was recorded in coir pith control bed (7.23±0.20 cm) followed by sugarcane control bed (7.0±1 cm), wood chips control bed (6.66±0.32 cm),

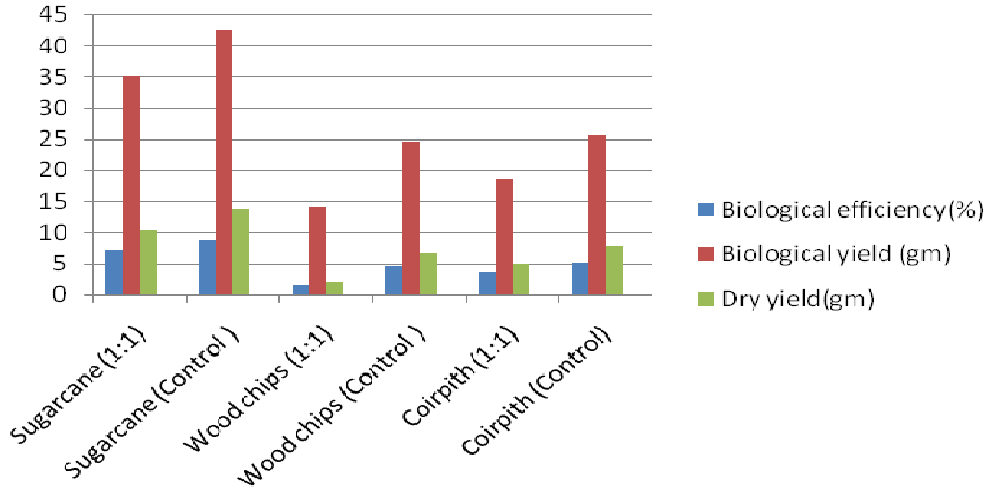

Figure 3. Effect of agro-industrial wastes on yield of *Pleurotus flabellatus*.

Figure 4. **(a)** Sugar cane: Cooked Fish Waste (1:1) bed, **(b)** coir pith: cooked fish waste (1:1) bed, **(c)** Woodchips: Cooked Fish Waste (1:1) bed.

sugarcane: cooked fish waste (1:1) (6.3±0.2 cm), coir pith: cooked fish waste (1:1) (6.26±0.20 cm), wood chips: cooked fish waste (1:1) bed (5.46±0.4 cm). The diameter of pileus is reported to increase the quality and yield of mushroom. The highest diameter of pileus was observed in mushroom grown on sugarcane control bed (5.43±0.15 cm), while the lowest value was recorded on coir pith: cooked fish waste (1:1) bed (4.26±0.15 cm) (Figure 2).

Biological efficiency (BE) and biological yield

The BE of oyster mushroom ranged from 4.87±0.02 to 11.63±0.02% on different substrates (Figure 3). The highest biological efficiency was recorded on control bag with sugarcane (11.63±0.02%) and the lowest BE was observed in wood chips: cooked fish waste (1:1) bed (4.87±0.02%). The maximum biological yield was recorded with control bag of Sugarcane (58.05±0.88 g) followed by sugarcane: cooked fish waste (1:1) (48.26±0.14 g) (Figure 4a), woodchips control (37.03±1.30 g), coir pith control (32.8±0.13 g), coir pith: cooked fish waste (1:1) (25.61±0.26 g) (Figure 4b). The lowest biological yield was observed in wood chips: cooked fish waste (1:1) (24.43±0.30 g) (Figure 4c).

Dry yield per packet

Dry yield of oyster mushroom grown on agro-industrial wastes varied from 4.24±0.11 to 19.33±0.11 g (Figure 3).

The highest dry yield was recorded on sugarcane control (19.33±0.11 g); the lowest dry yield was found on wood chips: cooked fish waste (1:1) (4.24±0.11 g).

DISCUSSION

Substrate is one of the important parameter in mushroom cultivation as mushrooms depend on substrates for nutrition to support mycelia growth and development into mushroom fruiting bodies. For the growth and penetration of the mycelium into basal substrates, which ultimately influences fruiting of mushrooms, the structure and porosity levels of substrate are important factors to be considered. (Mutemi Muthangya et al., 2014). In this study, days to complete mycelium running in mushroom bed ranged from 16 to 39 days on different agro-industrial waste, which was more or less similar 16-25 days of *Pleurotus ostreatus* reported by Shah et al. (2004) working on wheat straw, leaves and saw dust. Islam et al. (2009) also suggested that the total days required completing mycelium running in Mahogany, Jam, Shiris, Kadom, and Jackfruit sawdust was 25 days. While Mango and Coconut sawdust took 26 and 30 days respectively to complete mycelial running. On the other hand, Baysal et al. (2003) found the fastest spawn running (15.8 days) in waste paper as substrate. From the results obtained in this study, days to first harvest ranged from 23.33±1.52 to 47.33±0.57 on different substrate. The maximum number of days required from opening to first harvest was recorded with Coir pith: Cooked Fish Waste (1:1) bed (47.33±0.57). Similar results on *Pleurotus* sp. have been reported by Shah et al. (2004); Mutemi Muthangya et al. (2014). In this study, the biological yield was recorded with Coir pith: Cooked Fish Waste (1:1) bed (25.61±0.26g). In the present study the number of days required from opening to primordial initiation was on the Coir pith: Cooked Fish Waste (1:1) bed (44±1).The number of days required from opening to first harvest was recorded with Sugarcane: Cooked Fish Waste (1:1) bed (40.33±1.15). Tripathy et al. (2011); Mshandete (2008) observed that the duration of different growth stages of cultivated mushrooms are affected by several factors which would include, but not limited to, type of substrates and supplements used, the type of species and/or the strain employed, spawn type and the rate of inocula/spawn applied, spawning method, spawning /cropping containers as well as on the prevailing mushroom growing conditions. In this study, the dry yield of oyster mushroom grown on agro-industrial wastes varied from 4.24±0.11 to 19.33±0.11g. This result is similar to the findings of Ashrafuzzaman et al. (2009) who investigated the dry yield varied possibly due to the variation of chemical composition of different substrates. Recently, Lopez Castro et al. (2008) stated that *Pleurotus* waste was adequate to sustain the growth of *Salvia officinalis* by improving air porosity and mineral content of the soil. This research is to looking at the possible use of fish waste and agro-industrial wastes for mushroom cultivation, which would provide medicinal food and encourage the biological conversion processes of agro-industrial wastes. Composting fish waste is a relatively new, practical and an environmental sound alternative to disposing of fish waste. It is economical, fairly odorless and a biologically beneficial practice for seafood operations. The study has shown that composting is safe and a cost effective solution for the seafood industry.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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